

Foundational characterization of tomato fruit RALF peptides reveals structural and functional specialization within the SIRALF family

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Abstract

Rapid Alkalinization Factor (RALF) peptides regulate plant growth and cell wall signaling, but their roles in fruit development remain unclear. Here, we characterized tomato (*Solanum lycopersicum*) fruit-associated RALF peptides and their interactions with leucine-rich repeat extensins (LRXs). Expression analyses identified *SIRALF5*, *SIRALF7*, and *SIRALF10* as the main fruit-expressed RALFs. *SIRALF10* was associated with early fruit development, whereas *SIRALF5* and *SIRALF7* remained expressed during ripening. Sequence analyses showed that *SIRALF5/7* retain conserved motifs of canonical RALFs, while *SIRALF10* displays divergent structural features and altered charge distribution. Synthetic *SIRALF5* and *SIRALF7* inhibited root growth and induced extracellular alkalinization, whereas *SIRALF10* lacked both activities. Co-immunoprecipitation assays showed that all three peptides interact with the fruit-expressed proteins SILRX2 and SILRX5. Structural modeling predicted distinct electrostatic properties for the SILRX5/*SIRALF10* complex compared with *SIRALF5*. These results reveal structural and functional specialization among tomato fruit RALF peptides and suggest that distinct *SIRALFs* may differentially respond to cell wall remodeling during fruit development and ripening.

Introduction

RAPID ALKALINIZATION FACTOR (RALF) peptides constitute a family of cysteine-rich plant peptide hormones that are involved in a wide range of biological processes, including root growth, reproduction, immune responses, and adaptation to abiotic stress conditions (Campbell & Turner, 2017; Lu et al., 2025; Murphy & De Smet, 2014; Pratyusha & Sarada, 2025; Schade et al., 2025). RALF peptide precursors typically range from 50 to 250 amino acids in length and generally contain an N-terminal signal peptide that directs them into the secretory pathway, where they undergo proteolytic maturation mediated by Site-1 Protease (S1P), a subtilisin-like serine protease that recognizes a RRXL motif located upstream of the RALF active domain (Abarca et al., 2021; Blackburn et al., 2020; Pearce et al., 2010; Srivastava et al., 2009). RALF peptides have been identified in more than 50 plant species, underscoring their evolutionary conservation and functional relevance (Campbell & Turner, 2017; Murphy & De Smet, 2014; Zhang et al., 2020; 2023).

RALF peptides were initially identified as ligands for plasma membrane-localized *Catharanthus roseus* receptor-like kinases 1-like (CrRLK1Ls) proteins and

glycosylphosphatidylinositol (GPI)-anchored proteins of the LORELEI/LORELEI-like GPI-anchored protein (LRE/LLG) family (Solis-Miranda & Quinto, 2021; Zhu et al., 2021). More recently, RALF peptides have been shown to play dual structural and signaling roles in maintenance of cell wall integrity (Manchanda & Geitmann, 2025). RALFs interact with cell wall-associated receptors known as Leucine-rich repeat (LRR)-extensin (LRX) proteins. These peptides exhibit high affinity for the LRR domain under acidic conditions, whereas the extensin domain is covalently cross-linked to the wall matrix (Herger et al., 2019; Moussu & Ingram, 2023). The C-terminal region of RALFs binds to LRX proteins, exposing positively charged cationic residues that physically interact with de-methylesterified, negatively charged pectins, thereby forming supramolecular assemblies that contribute to cell wall integrity during plant development (Moussu et al., 2023; Schoenaers et al., 2024). In *Arabidopsis*, the RALF4-LRX8 complex binds specifically to de-methylesterified homogalacturonan (HG) in a charge-dependent manner, thereby influencing cell wall organization during pollen tube growth (Moussu et al., 2023). Similarly, the RALF22-LRX1/2 complex organizes de-methylesterified HG into circumferential rings at the root hair tip, modulating cell wall architecture (Schoenaers et al., 2024).

De-methylesterified pectins promote cell wall loosening and increase cell wall permeability, processes associated with cell expansion and elongation in structures such as root hairs and pollen tubes. Given that fruit ripening also involves extensive de-methylesterification and remodeling of HG, similar RALF-mediated mechanisms may operate in fruit tissues. Pectins are particularly abundant in fruit cell walls, accounting for up to 60% of the cell wall dry weight, and their remodeling is a key feature associated with fruit softening (Brummell, 2006; Hyodo et al., 2013; Posé et al., 2019; Shi et al., 2023). Fruit softening is generally characterized by highly coordinated pectin remodeling. HG is secreted into the apoplast as long chains in a highly methyl-esterified state. Pectin methylesterases (PMEs) are typically upregulated during ripening; for example, in tomato, pectins are approximately 90% methyl-esterified in mature green fruit, decreasing to around 35% in red-ripe fruit (Hyodo et al., 2013; Jeong et al., 2018). PMEs can act in a random manner, rendering HG more susceptible to degradation by pectinolytic enzymes such as polygalacturonase (PG) and pectate lyase (PL) (Palin & Geitmann, 2012). Consistently, downregulation of PG or PL genes has been shown to enhance shelf life and/or reduce fruit softening in tomato, strawberry, apple and peach (Atkinson et al., 2012; Paniagua et al., 2016; Qian et al., 2021; Santiago-Doménech et al., 2008; Uluisik & Seymour, 2020; Wang et al., 2018). Understanding the regulatory mechanisms underlying pectin remodeling is therefore of major agronomic interest for improving fruit firmness and postharvest shelf life.

The involvement of RALF peptides in pectin remodeling during fruit development has received limited attention (Huang et al., 2024; Montano et al., 2026). One of the few studies available was conducted in strawberry, where silencing the expression of *FaRALF29* delayed fruit colonization by *Colletotrichum* species, pathogens associated with fruit rot during storage and transport (Cesarino, 2023). In contrast, overexpression of the *FaRALF29* gene in unripe white fruits induced disease symptoms (Merino et al., 2019). We hypothesized that fruit-expressed RALF peptides regulate pectin remodelling and contribute to fruit softening during tomato ripening. Transcriptomic analyses

identified *SIRALF5*, *SIRALF7* and *SIRALF10* as preferentially expressed during fruit development. In this study, as a first step towards understanding their role, we characterized them through physiological and biochemical approaches.

Results

SIRALF and *SILRX* gene expression pattern analysis

The tissue-specific expression of tomato *CrRLK1L* genes has been reported by Ma et al., 2023. Among the 24 *SiCrRLK1L* genes, three showed high expression in fruit tissues: *SiCrRLK1L20* was the most highly expressed, reaching its maximum at the breaker (Br) plus a 10-day fruit stage; *SiCrRLK1L15* exhibited a similar pattern to *CrRLK1L20*, but with lower expression levels; and *CrRLK1L7* showed a moderate expression in fruit tissue, peaking at the Br stage and decreasing at Br+10 (Ma et al., 2023). To investigate the tissue-specific expression of tomato *SIRALF* and *SILRX* genes, we followed a similar approach. The expression profiles of all the 11 *SIRALF* genes and 5 *SILRX* genes were analyzed in the following tissues: root, leaf, bud, flower, 1-3 cm fruit, mature green fruit, breaker fruit, and breaker plus a 10-day fruit. The original RNA-seq data were obtained from the SGN tomato functional genomic database (SGN-TFGD, <http://ted.bti.cornell.edu/cgi-bin/TFGD/digital/home.cgi>) (Sato et al., 2012). As shown in Figure 1a, *SIRALF5* and *SIRALF7* genes were predominantly expressed at the mature fruit stage. *SIRALF9* and *SIRALF10* were expressed up to mature green stage, after which their expression declined. The remaining *SIRALF* genes showed little or no expression in fruit tissue, appearing to be specific to other organs, for example *SIRALF2* in roots, and *SIRALF1*, *SIRALF3*, *SIRALF6*, *SIRALF8* in buds and flowers. As shown in Figure 1b, two *SILRX* genes were expressed in fruit tissue: *SILRX5* was the most highly expressed, peaking toward the end of ripening, while *SILRX2* exhibited lower expression levels. *SILRX1* and *SILRX3*, in contrast, were mainly expressed in flowers. Based on these expression pattern, we hypothesize that *SIRALF5* and *SIRALF7* represent candidate regulators during the ripening process, whereas *SIRALF9* and *SIRALF10* may function during cell expansion phase up to the mature green stage.

SIRALF5 and *SIRALF7* cluster within the same group as Arabidopsis RALF1, RALF22, RALF23 and RALF33, which also includes most fruit-expressed RALFs, such as *FaRALF1* and *FaRALF39*, both of which show the highest expression levels in mature strawberry fruits (Montano et al., 2026; Negrini et al., 2020). In contrast, *SIRALF9* and *SIRALF10* clusters within a less well-supported group together with Arabidopsis RALF27 (Montano et al., 2026). Notably, *SIRALF10* was not included in the classification proposed by (Campbell & Turner, 2017). To investigate the relationship between Arabidopsis and tomato LRX proteins, a phylogenetic analysis was performed using full-length amino acid sequences (Supplementary Figure 1). *SILRX5*, which exhibits the highest expression during fruit ripening (Figure 1b), groups together with Arabidopsis LRX5, expressed in roots and shoots, and LRX6, which is associated with lateral root formation (Supplementary Figure 1) (Herger et al., 2019). *SILRX2*, which also shows moderate expression during fruit ripening, cluster with Arabidopsis LRX1 and LRX2, both predominantly expressed in root hairs (Supplementary Figure 1) (Herger et al., 2019).

Conserved motifs in the mature forms of *SIRALF5*, *SIRALF7* and *SIRALF10* proteins expressed in tomato fruit

To investigate the role of RALFs in fruit formation, we selected *SIRALF5* and *SIRALF7* as candidates of interest, as they are expressed during fruit ripening, and *SIRALF10*, which shows high expression from the initial stages of fruit development until the mature green stage (Figure 1a). Phylogenetic analysis revealed that *SIRALF5* and *SIRALF7* proteins share high sequence similarity with *SIRALF2*, which is expressed in roots (Montano et al., 2026) (Figure 1a). These three *SIRALF* peptides group together with *Arabidopsis* RALF1, RALF22, RALF23 and RALF33 (Montano et al., 2026). We have also included *Arabidopsis* RALF4 and RALF19, since they have been well characterized with respect to amino acid motifs important for their function (Moussu et al., 2020; 2023; Schoenaers et al., 2024; Xiao et al., 2019). *SIRALF9* and *SIRALF10* cluster with *Arabidopsis* RALF27 (Montano et al., 2026). We aligned all mature form from protein sequences to compare them with the motifs described in the literature (Figure 2).

SIRALF2, *SIRALF5* and *SIRALF7* are classified as true RALFs, as their sequences contain the RRXL cleavage site, the YISY motif, and the other well conserved motifs GASYY, PYXRGCS and RCRR(S) (Figure 2) (Bedinger et al., 2010; Campbell & Turner, 2017). The YISY motif is essential for RALF1-induced alkalization, together with the Leu-11 residue, whose deletion was reported to have a profound effect on alkalization activity (Pearce et al., 2010). *SIRALF2*, *SIRALF5* and *SIRALF7* conserve this Leu residue, whereas *SIRALF9* and *SIRALF10* do not (Figure 2). Residues 4-17 at the N-terminal of RALF23 establish extensive interactions with LLG2, where only the YISY motif is required to nucleate the ternary complex LLG-RALF-FER (Xiao et al., 2019). The N-terminal residues of RALF23 reported to form polar interactions with LLG2 are conserved in *SIRALF2*, *SIRALF5* and *SIRALF7* (Figure 2). However, the basic Arg residues surrounding the YISY motif in RALF23, important for polar interaction with LLG2, are not fully conserved. *SIRALF2* lacks these residues entirely, while in *SIRALF5* and *SIRALF7* some are replaced by polar amino acids (Figure 2). Among the other *Arabidopsis* RALFs (RALF1, RALF4, RALF19, RALF22 and RALF33), these residues are largely conserved, with only minor substitutions in some cases. *SIRALF10* possesses an equivalent YLSY motif, similar to *Arabidopsis* RALF27, whereas *SIRALF9* lacks the YISY/YLSY motif entirely (Figure 2). The basic Arg residues surrounding the YISY/YLSY motif are also absent in *SIRALF10*, making it more similar to *SIRALF2*.

Analysis of the RALF4-LRX8 interface revealed that LRX proteins recognize the mature sequence, and the YISY motif is shown to be unnecessary for binding (Moussu et al., 2020). All four cysteines in RALF4 were required for proper peptide folding and high affinity binding to LRX8 (Moussu et al., 2020). These cysteines are conserved across all five *SIRALF* peptides (Figure 2). Two loops formed by the cysteine pairs are critical for LRX8 recognition (Moussu et al., 2020). The first loop is highly conserved among *SIRALF2*, *SIRALF5* and *SIRALF7* and related *Arabidopsis* RALFs. It contains two tyrosine residues that directly contact the LRR core (Figure 2) (Moussu et al., 2020). These tyrosines are conserved in all analyzed RALFs except RALF27 and *SIRALF9*, where nonpolar residues occur, and in *SIRALF10*, where one tyrosine is replaced by a Glycine (Figure 2). Apart from a few charged residues specific to RALF4 and RALF19, this loop is otherwise conserved. The second shows a similar pattern, with a single charge residue unique to RALF4 and RALF19 (Moussu et al., 2020). Both loops are absent in *SIRALF9* and *SIRALF10* (Figure 2).

Between the two cysteines loops, RALF4 exhibits an exposed, positively charged surface when bound to LRX8, composed by residues Arg32-34 (89-91) in a protruding loop and

extending to Lys13 (70), Arg21 (78), Lys30-31 (87-88) and Arg39 (96). Numbers in brackets are from the N-terminal part of the full prepropeptide according to (Moussu et al., 2020; 2023). This basic patch mediates electrostatic interaction with pectin (Moussu et al., 2020; 2023). Positively charged residues at positions 4-5 (61-62), located immediately upstream of the YISY motif, are conserved in RALF22, RALF23, RALF19, RALF27, SIRALF5 and SIRALF7, but absent in SIRALF9 and SIRALF10 (Figure 2). Lys13 (70) is not conserved in SIRALF2, SIRALF5 or SIRALF7, whereas Arg21 (78) is fully conserved (Figure 2). The main positively charged patch in RALF4 (residues 30-34) is fully conserved only in RALF19; in other RALFs, these residues are replaced by polar and nonpolar amino acids (Figure 2). While RALF22 retains only one Arg of this positively charged patch, most of the other basic residues are conserved with RALF4, including three C-terminal Arg residues, but within positions 30-34 (87-91) only one Arg remains (Schoenaers et al., 2024). SIRALF5 and SIRALF7 show a pattern similar to RALF22, except that Arg82 and Arg100 are not conserved (Figure 2). The three residues Arg12 (82), Arg20 (90) and Arg30 (100) are exposed when RALF22 binds to LRX, mediating the interaction with demethylesterified oligogalacturonide (Schoenaers et al., 2024). Overall, the LRX-binding surface of RALF4 and RALF22 is conserved among closely related RALFs, including SIRALF2, SIRALF5 and SIRALF7, but diverges markedly in RALF27, SIRALF9 and SIRALF10 (Figure 2) (Moussu et al., 2023.; Schoenaers et al., 2024). Despite this divergence, these three RALFs retain several positively charged residues at comparable positions (Figure 2). Notably, SIRALF10 contains six acidic amino acids distributed along its sequence, whereas most Arabidopsis RALFs lack acidic residues in their mature sequence, with the exception of RALF4, RALF19 and RALF27, which each contain one or two (Figure 2).

SIRALF5/7/10 physiological responses in *A. thaliana*

One of the described functions of Arabidopsis RALF peptides is their ability to inhibit cell expansion and growth, as well as to alkalinize the extracellular medium. Most exogenously applied RALF peptides are able to induce root growth inhibition and alkalinization in Arabidopsis suspension cultures (Abarca et al., 2021; Blackburn et al., 2020; Morato do Canto et al., 2014). Here, we screened SIRALF peptides expressed in tomato fruits at higher levels, SIRALF5, SIRALF7 and SIRALF10, for their bioactivity using a root growth inhibition assay in Arabidopsis. Five day-old seedlings were treated with synthetic SIRALF peptides (Supplementary Table S3) for two days before measuring root length. SIRALF5 and SIRALF7 significantly inhibited root growth in three independent biological experiments, whereas SIRALF10 did not (Figure 3a). In a further assay, SIRALF5 caused significant inhibition of seedling growth in Arabidopsis, whereas SIRALF10 showed no effect (Supplemental Figure 2a).

We performed alkalinization assays using a customized real-time pH monitoring system to quantitatively measure extracellular alkalinization in tomato cell suspension culture under response to SIRALF peptides (Wang et al., 2024). SIRALF5 and SIRALF7 clearly induced medium alkalinization, while SIRALF10 did not (Figure 3b). Based on these results, we selected SIRALF5 and SIRALF10 for further assays assessing root growth inhibition in tomato. Once again, SIRALF5 caused significant inhibition of root growth, whereas SIRALF10 showed no effect (Supplemental Figure 2b). These results confirm that alkalinization and growth inhibition are coupled characteristics of certain RALF peptides (Abarca et al., 2021). Finally, we investigated whether these tomato fruit RALF

peptides trigger reactive oxygen species (ROS) production. Several Arabidopsis RALF peptides have previously been reported to induce significant ROS accumulation (Abarca et al., 2021). None of the SIRALF peptides tested were able to induce a significant ROS response (Supplementary Figure 2c).

SIRALF5/7/10 interact with SILRX proteins

In Arabidopsis, several RALFs have been reported to physically interact with LRX and/or CrRLK1L receptor proteins. For example, RALF4 binds to the CrRLK1L proteins BUPS1/2 and ANX1/2, and in a mutually exclusive manner to the LRR domain of LRX8, thereby regulating pollen tube integrity (Ge et al., 2017; Mecchia et al., 2017; Moussu et al., 2023). To examine whether SIRALF5, SIRALF7 and SIRALF10 peptides directly interact with LRX receptor proteins (SILRX2, SILRX5), coimmunoprecipitation (Co-IP) assays were performed. The LRR domains of SILRX proteins were fused to a green fluorescent protein (GFP) tag, and SIRALF peptides were fused to a double hemagglutinin (HA) tag in *Agrobacterium*-infiltrated *Nicotiana benthamiana* leaves. Immunoprecipitation was conducted using GFP-Trap agarose beads. The three mature SIRALF-HA peptides (SIRALF5, SIRALF7 and SIRALF10) were detected when co-expressed with either SILRX2-GFP or SILRX5-GFP (Figure 4). Note that RALF propeptide and mature peptide forms are detected as previously reported (Mecchia et al., 2017).

Based on these results, we scrutinized LRR domain sequences of SILRX2 and SILRX5 for the presence of sequence motifs previously reported to mediate the interaction of Arabidopsis LRX proteins with RALF peptides (Moussu et al., 2020). To this end, the LRR domain of SILRX2 was aligned with its closest Arabidopsis homologs, LRX1 and LRX2, which exhibited 63,1% and 64,8% sequence identity, respectively. Similarly, the LRR domain of SILRX5 was aligned with LRX3, LRX4 and LRX5, showing 72,6%, 72,4% and 69,2% sequence identity, respectively (Supplementary Figure 3). All five pairs of Cys residues previously described to form intra-molecular disulfide bonds, as well as the Cys residue responsible for the inter-molecular disulfide bridge covalently linking the two LRX protomers in Arabidopsis LRXs, are fully conserved in SILRX2 and SILRX5 (Supplementary Figure 3) (Moussu et al., 2020). We subsequently analyzed in detail the amino acids residues constituting the LRX-RALF4 binding pocket (Moussu et al., 2020). Within the described secondary structure, particularly in the β 3 and β 5 loops located between the first Cys dimer and the inter-protomer Cys dimer, SILRX5 displays an enrichment in polar residues relative to most Arabidopsis LRXs, with the exception of LRX7, which also exhibits a similar enrichment (Supplementary Figure 3) (Moussu et al., 2020). Overall, the high conservation of the RALF-binding pocket suggests that SILRX2 and SILRX5 may interact with RALF peptides in a conformation analogous to that characterized for Arabidopsis LRXs (Moussu et al., 2020).

Prediction of the SIRALF5/10 mature peptide interaction with SILRX5

Next, we modelled the SILRX5-SIRALF5 and SILRX5-SIRALF10 complexes using AlphaFold2, including the LRR domain and the mature SIRALF peptides (Figure 5). SILRX5 is structurally related to the LRX proteins LRX2 and LRX8 described in Arabidopsis (Lee & Santiago, 2023; Moussu et al., 2020). Consistent with experimentally determined LRX-RALF structures, SIRALF peptides adopt a folded, disulfide bond-stabilized conformation that fits into the conserved binding pocket within the LRR domain through extensive contacts. Our model predicts that SIRALF5 binds SILRX5 in a configuration compatible

with that described for Arabidopsis LRX-RALF. In this orientation, residues corresponding to R82, R90 and R100 of Arabidopsis RALF22 align with K78, R85 and K94 in SIRALF5 and remain solvent-exposed, consistent with the polycationic pectin-interaction surface in Arabidopsis (Figure 5a) (Moussu et al., 2020; Schoenaers et al., 2024). In contrast, the predicted SILRX5-SIRALF10 complex displays a distinct peptide conformation within the binding pocket. Although residues corresponding to the conserved polycationic surface (K62, K69 and K78 in SIRALF10) are present, their proximity to a negatively charged residue is predicted to alter their orientation and potentially modify the electrostatic surface of the peptide (Figure 5b).

Discussion

RALF peptides have been extensively studied in root development, plant reproduction, abiotic stress responses, and immunity (Liu et al., 2024; Lu et al., 2025). In contrast, their roles during fruit development and ripening remain poorly understood. Here, we provide an initial framework linking SIRALF peptides to tomato fruit biology. Tissue-specific expression analyses revealed three major expression patterns among *SIRALF* genes (Figure 1a). SIRALF1, SIRALF3, SIRALF6, and SIRALF8 are predominantly expressed in floral tissues, whereas SIRALF2 is mainly expressed in roots. By contrast, SIRALF5, SIRALF7, SIRALF9, and SIRALF10 show broader expression profiles. In fruit tissues, these four genes are expressed at the mature green stage, with SIRALF10 displaying the highest transcript abundance. However, only SIRALF5 and SIRALF7 remain expressed throughout ripening, suggesting specialized functions during late fruit development.

Phylogenetic analyses further support functional diversification within this family. SIRALF2, SIRALF5, and SIRALF7 cluster with Arabidopsis RALF1/RALF22/RALF23/RALF33, whereas SIRALF9 and SIRALF10 group with Arabidopsis RALF27 (Montano et al., 2026). Because RALF27 remains functionally uncharacterized, the analysis of SIRALF10 may also provide insights into the role of this poorly understood clade. Structural analyses revealed substantial differences between SIRALF10 and SIRALF2/5/7. Most SIRALF peptides are enriched in basic amino acids, but SIRALF2 and SIRALF10 are notable exceptions, displaying acidic pI values (Wang et al., 2025). SIRALF10 also shows a markedly different charge distribution compared with canonical RALFs such as Arabidopsis RALF4 (Figure 2) (Moussu et al., 2023). In addition, motifs associated with LLG interaction are highly conserved in SIRALF2/5/7 but only partially retained in SIRALF10. The GASYY and PYXRGCS motifs are similarly conserved in SIRALF2/5/7 but diverge in SIRALF9/10. Together, these observations suggest that SIRALF10 interacts with extracellular partners through mechanisms distinct from those of SIRALF2/5/7.

Differences in putative LRX interactions further support this idea. Two conserved Tyr residues stabilize RALF binding to the LRX pocket (Moussu et al., 2020), yet SIRALF10 retains only one of them (Figure 2). This feature may weaken LRX association and favor a more dynamic equilibrium between LRX-bound and LLG-FER-associated states. Moreover, residues involved in pectin interaction are strongly conserved in SIRALF2/5/7 but not in SIRALF10. In Arabidopsis, RALF4 and RALF22 interact electrostatically with demethylesterified pectin through positively charged surfaces (Moussu et al., 2023; Schoenaers et al., 2024). By contrast, SIRALF10 contains negatively charged residues

within the predicted interaction interface, which may alter its affinity for pectin or favor binding to specific methylesterification status.

These structural differences support a model in which distinct SIRALF peptides could respond differently to ripening-associated changes in the apoplast. During tomato ripening, pectin demethylesterification and degradation progressively increase the abundance of negatively charged oligogalacturonides (OGs) (Wang & Seymour, 2022). Because SIRALF5/7 retain highly conserved positively charged surfaces, these peptides may become increasingly sequestered by OGs through electrostatic interactions. Such sequestration could reduce their availability for CrRLK1L receptor binding and attenuate downstream signaling. In contrast, SIRALF10 may operate through a more dynamic interaction regime that depends on pH, cell wall status, and the balance between LRX- and LLG/CrRLK1L-associated complexes. This functional diversification may provide a mechanism linking cell wall integrity with receptor-mediated signaling during fruit ripening. These contrasting structural properties of SIRALFs are consistent with their distinct biological activities. SIRALF5/7 inhibited root growth and induced alkalization of the growth medium, whereas SIRALF10 did not (Figure 3 and Supplementary Figure 2). Similar phenotypes have been reported for Arabidopsis RALF27, which also lacks growth inhibitory and alkalization activity (Abarca et al., 2021). These observations support the idea that SIRALF10 belongs to a functionally distinct subgroup. Although several RALF peptides induce ROS accumulation (Abarca et al., 2021), neither SIRALF5 nor SIRALF10 triggered significant ROS production under our experimental conditions (Supplementary Figure 2).

SLCrRLK1L20, the closest tomato homolog of Arabidopsis FER, is highly expressed during fruit ripening and positively regulates both ripening progression and resistance to *Botrytis cinerea* (Ji et al., 2020, 2023; Ma et al., 2023). Because SIRALF5 and SIRALF7 interact with SLCrRLK1L20ECD in yeast two-hybrid assays (Wang et al., 2025), these peptides may function upstream of FER-like signaling pathways during ripening. This raises the possibility that RALF-mediated sensing of cell wall status contributes to the coordination of ethylene signaling, defense responses, and fruit softening. In parallel, our results identify LRX proteins as additional components of this regulatory system. SLRX5 and, to a lesser extent, SLRX2 are expressed throughout fruit development and temporally overlap with SIRALF5/7/10 expression (Figure 1b). Co-IP assays confirmed interactions between these peptides and both SLRX proteins (Figure 4), supporting the existence of LRX–RALF complexes in tomato fruit. Among them, SLRX5 likely plays the predominant role. Its interaction with SIRALF10 may be especially relevant during early fruit development, whereas SIRALF5/7 interactions may predominate during ripening.

Structural modeling further supports differential interaction modes among SIRALFs. The predicted SLRX5–SIRALF5 complex closely resembles experimentally characterized Arabidopsis RALF–LRX structures and retains a positively charged binding surface compatible with pectin interaction (Figure 5A). By contrast, the predicted SLRX5–SIRALF10 complex displays a less positively charged interface due to the presence of exposed acidic residues (Figure 5B). Although these predictions require experimental validation, they support the idea that distinct SIRALF peptides could engage cell wall components through different electrostatic interaction patterns. These observations fit

well with the extensive cell wall remodeling that accompanies fruit ripening. Pectin depolymerization and demethylesterification progressively weaken the wall and increase apoplastic porosity (Posé et al., 2019; Wang & Seymour, 2022). PME activity initially generates demethylesterified homogalacturonan domains that can transiently reinforce the wall through Ca^{2+} -mediated crosslinking, but subsequent degradation by pectate lyase and polygalacturonase ultimately drives irreversible softening (Peng et al., 2022; Sénéchal et al., 2014; Uluisik & Seymour, 2020). Within this dynamic environment, RALF–LRX–FER modules may act as sensors that translate changes in pectin status and apoplastic pH into signaling outputs that coordinate wall remodeling, ripening progression, and defense responses. Collectively, our results provide a theoretical model in which distinct SIRALF peptides differentially may signal ripening-associated cell wall remodeling through interactions with LRX and CrRLK1L proteins. Further functional studies will clarify how this signaling network is linked to pectin status, apoplastic pH, and receptor-mediated pathways to coordinate fruit softening during tomato ripening.

Materials and Methods

Gene expression pattern and phylogenetic analyses

The *SIRALF* and *SILRX* gene expression pattern analyses used RNA-seq data from the SGN tomato functional genomic database (SGN-TFGD, <http://ted.bti.cornell.edu/cgi-bin/TFGD/digital/home.cgi>) (Sato et al., 2012). The data are listed in Tables S1 and S2. The heatmap was illustrated with Morpheus (<https://software.broadinstitute.org/morpheus/>). The RALF and LRX proteins sequences from tomato and Arabidopsis were downloaded from the National Center for Biotechnology Information (NCBI, <https://www.ncbi.nlm.nih.gov/>) and TAIR (<https://www.arabidopsis.org/>), respectively. The obtained full protein sequences were aligned by ClustalW to construct a phylogenetic tree with the default parameters. Multiple sequence alignment of the mature peptide sequence were created using ClustalW.

Plant material and growth conditions

We used *A. thaliana* (Col-0 ecotype), *S. lycopersicum* (Money Maker) and *N. benthamiana* as experimental materials. Arabidopsis and tomato seeds were surface sterilized with chlorine vapors (100 mL commercial bleach + 3 mL 37% HCl) in a sealed container for 3-4h, then air-cleared for 2-4h in a laminar flow cabinet. Seeds were plated under sterile conditions on ½ Murashige-Skoog medium with 1% (w/v) sucrose and 1% (w/v) agar. Plated seeds underwent 3-d vernalization at 4°C in darkness, then grew vertically in a chamber (16h light/8h dark, 130 +/- 30 μmol photons m⁻² s⁻¹, 22 +/- 1°C).

Peptides

SIRALF peptides were synthesized (Supplemental Table S3) by GenScript Biotech (www.genscript.com) with a purity of >90%. The peptides were dissolved in sterile pure water for usage and stored at -20°C at a concentration of 1 mM.

Root growth inhibition assay

Arabidopsis and tomato seeds were grown for 5 d before transferring ten seedlings to each well of a 12-well plate containing 4 mL per well of ½ MS medium containing 2 µM RALF. Seedlings were transferred 2 d later to solid ½ MS plates. Control seedlings were performed under identical conditions in a peptide-free medium. Primary root length was measured by scanning the plates and quantified using the software Fiji (<https://imagej.net/Fiji>). Experiments were repeated at least three times using independent biological replicates.

Seedling growth inhibition assay

Arabidopsis seeds were grown for 5 d before transferring ten seedlings to each well of a 12-well plate containing 4 mL per well of ½ MS medium containing 2 µM RALF. Seedlings weight was measured 7 d later. Control seedlings were performed under identical conditions in a peptide-free medium. Experiments were repeated at least three times using independent biological replicates.

Alkalinization assay

Extracellular alkalinization assays were performed in tomato (*Solanum peruvianum*) wild type suspension cultures as described (Wang et al., 2024; Li et al., 2025). Briefly, RALF peptides were diluted and added into 10 mL of seven-day old cell suspension from 200-fold concentrated stock solutions prepared in ddH₂O. Following elicitor treatment, pH of treated cells was recorded at 10s intervals for 60 mins. Dosage curve was then plotted using peak values at each concentration. At least three biological replicates were performed for each treatment.

ROS production assay

Arabidopsis seedlings were sterilized and vernalized as described above. Plates were transferred to short day photoperiod (8h light/16h dark) for 4-5 days. After this period, plants were transferred to soil composed of organic substrate and vermiculite (4:1 v/v) during four weeks under short day. ROS measurement was performed as previously described (Sang and Macho, 2017). Plant discs were incubated overnight with water. The next day, water was replaced by 75 µL 2 mM MES-KOH pH 5.8 to mimic the apoplastic pH. Leaf discs were incubated for 4h before adding 75 µL 40 µg/mL horseradish peroxidase (HRP), 1 µM L-O12 (Wako Chemicals) and 4 µM RALF peptide. In total, 24 leaf discs were used for each condition. Luminescence was measured using a GloMax 96 Microplate Luminometer (Promega).

Plasmid constructs

SILRX2 and *SILRX5* were amplified from *S. lycopersicum* genomic DNA with high-fidelity DNA polymerase (Q5 High-Fidelity DNA Polymerase, New England Biolabs, #M0491). *SILRX* genes were cloned until the Cys-rich region end predicted in (Moussu S et al., 2020). All the primer sequences are listed in Supplementary Table S4. Using Multisite Gateway cloning, the PCR product were inserted into pDONR L1-L2 via BP reactions (Invitrogen). All constructs were verified by PCR, restriction analysis and sequencing. The sequences encoding SIRALF peptides, together with sequenced coding for a 2xHA-epitope tag flanked by att sites (Gateway, Life Technologies), were synthesized using Genscript technology (Genscript, NJ, USA): SIRALF5 (5' CAAATAATGATTTTATTTTGACTGATAGTGACCTGTTGTTGCAACACATTGATGAGCAATGCT

TTTTATAATGCCAACTTTGTACAAAAAAGCAGGCTCCATGGGAGTTTCTTCGTATTTGATTGTT
TGTGTTCTTGTTGGAGCTTTTTTTCATTTCCATGGCTGCCGCCGGCGACAGTGGTAGCTACGATT
GGATGGTGCCGGCGAGATCCGGTGAATGCAAGGGGAGTATTGCAGAGTGCATGGCTGAAGA
AGATGAGTTTGCCCTTGACAGTGAGTCAAACAGGCGTATTTTAGCAACCAAAAAGTACATCAG
CTATGGTGCACTCCAGAAGAACAGTGTGCCGTGTTCTCGCCGTGGAGCTTCCTACTACAACTG
CAAACCTGGAGCTCAAGCAAATCCCTACACTCGTGGATGCAGTGCTATTACTCGTTGCAGGAG
CTACCCATACGATGTTCCAGATTACGCTTACCCATACGATGTTCCAGATTACGCTTAGCACCCA
GCTTTCTTGACAAAAGTTGGCATTATAAGAAAGCATTGCTTATCAATTTGTTGCAACGAACAGG
TCACTATCAGTCAAATAAAAATCATTATTTG3'), SIRALF7
(5'CAAATAATGATTTTATTTTGACTGATAGTGACCTGTTTCGTTGCAACACATTGATGAGCAATG
CTTTTTTATAATGCCAACTTTGTACAAAAAAGCAGGCTCCATGGCGAAAGTGAAATCTCTCTCT
ATTTTCTTCATCTCATCCATTTTCTCCGTCGTTATTGTAGCGCTTTTATCTCCGGCCGCGCTGGT
TCCGCGGCGGCAGTAGCTGCTACTGGGTCCACCAGCTGAGTTACTTTCCGATGACATTATCTT
CTTCTTCTTCACCGATCTGCGACGGTTCGATCGGGGACTGTTTGGCTGAAGAAGACGAGAATG
AGTTCGGGATGGAATCGGAGAGCAGCCGGCGCATGTTAGCATACCGCCGGAGATACATTAGC
TACGGCGCGCTTAGTAGTAACAGAGTGCCGTGTTCAAGGAGAGGAGCTTCGTAATAAATTG
CCGTCCCGGAGCTCAGGCGAATCCTTATCAACGTGGATGCAGTGCCATCACGCGCTGCCGTCA
TTACCCATACGATGTTCCAGATTACGCTTACCCATACGATGTTCCAGATTACGCTTAGCACCCA
GCTTTCTTGACAAAAGTTGGCATTATAAGAAAGCATTGCTTATCAATTTGTTGCAACGAACAGG
TCACTATCAGTCAAATAAAAATCATTATTTG3') and SIRALF10 (5'
CAAATAATGATTTTATTTTGACTGATAGTGACCTGTTTCGTTGCAACACATTGATGAGCAATGCT
TTTTTATAATGCCAACTTTGTACAAAAAAGCAGGCTCCATGAGGAGCTGCTGCAGTTTGTGTTG
GCTATCCATGGTGATTTTGTCTCTGCTTCTTCGGTAACAGACGGGACGATGATCGTCGAAAG
GCGGTGGAACGGCACCGGAGAAATGGATGGTATTGATTGGGAGGTTAGCCTTGCAGGTGAT
GTAAATTACTTGTCTTACAAAGTTACTAATGATAAGCCACCGATCTGCAACGAGAAAGTGTAC
GGGAATTGCATAGGGGACAAGAAGCCTGGCCAACGCCCATGTACTGAATACAATAACTGCAA
GCGTTCTCCAGATTACCCATACGATGTTCCAGATTACGCTTACCCATACGATGTTCCAGATTAC
GCTTAGCACCCAGCTTTCTTGACAAAAGTTGGCATTATAAGAAAGCATTGCTTATCAATTTGTT
GCAACGAACAGGTCATCAGTCAAATAAAAATCATTATTTG3').

For expression plasmid generation, the entry vectors (pENTRY L1-Gene-L2) were recombined with destination vectors using a LR Clonase™ reaction (Invitrogen). Specifically, pGWB5 was used for receptor constructs and pGWB2 for peptide constructs. The resulting plasmids are 35S:SILRX2-GFP, 35S:SILRX5-GFP; and 35S:SIRALF5-HA, 35S:SIRALF7-HA and 35S:SIRALF10-HA.

Transient expression in *N. benthamiana*

The binary plasmids were transformed into *A. tumefaciens* strain GV3101::pMP90 by electroporation. *A. tumefaciens* strains were grown overnight at 28°C in LB medium with rifampicin (50 µg/mL), gentamicin (25 µg/mL), and the construct specific antibiotic kanamycin (50 µg/mL). Cultures were centrifuged (3,000 x g, 15 min, RT), and pellets were resuspended in agroinfiltration solution (10 mM MES pH5.6, 10 mM MgCl₂, 1 mM acetosyringone), then incubated in the dark for 2-4h at RT. For double-gene expression, *Agrobacterium* suspensions were mixed to reach OD₆₀₀ of 0.33 per constructs and 0.25 for p19, to prevent gene silencing. Two leaves from 3-week-old *N. benthamiana* plants (3rd and 4th from the apex) were infiltrated on the abaxial side using a needleless 1 mL

syringe. Plants were maintained under growth conditions for 2 d before sample collection.

Co-immunoprecipitation assay and immunoblot analysis

Co-IP experiments were conducted based on the methodology described in (Amorim-Silva et al., 2019). *N. benthamiana* leaves transiently transformed were ground in liquid nitrogen to a fine powder. Proteins were extracted from 0.5 g of tissue by incubating the ground material for 30 min at 4°C under rotation with 1 mL of nondenaturing extraction buffer (150 mM Tris-HCl pH 7.5, 150 mM NaCl, 1% (v/v) Nonidet P-40, 10 mM EDTA, 1 mM Na₂MoO₄, 1 mM NaF, 10 mM DTT, 0.5 mM Pefabloc[®], 1% (v/v) protease inhibitor cocktail (Sigma, #P9599)). The resulting extracts were centrifuged (16,000 x g, 20 min, 4°C) and filtered through Poly-Prep chromatography columns (Bio-Rad, #731-1550). An aliquot of 100 µL was set aside as input for Western blot analysis, while the remaining extract was incubated for 2h with 30 µL GFP-Trap[®] Magnetic Agarose beads (Chromotek). To reduce unspecific interactions, Nonidet P-40 concentration was adjusted to 0.2% during incubation. Beads were washed four times with detergent-free extraction buffer, then resuspended in 75 µL of 2 x Laemmli Buffer (125 mM Tris-HCl pH6.8, 4% (w/v) SDS, 20% (v/v) glycerol, 2% (v/v) β-mercaptoethanol, 0.01% (w/v) bromophenol blue). Samples were heated at 95°C for 5 min, and subsequently centrifuged (20,000 x g, 1 min RT) to dissociated immunocomplexes. Proteins extracts were separated by molecular weight using 10% SDS-PAGE. Electrophoresis was carried out at a constant voltage of 80 V during stacking, followed by 120 V for protein separation. Subsequently, proteins were transferred onto a methanol-equilibrated PVDF membrane (Immobilon-P, Millipore, 0.45 µm, #IPVH00010) using a semi-dry transfer method following the standard protocol of the Trans-Blot Turbo Transfer System (Bio-Rad), at 25 V for 30 min. Membranes were then blocked with 5% (w/v) skimmed milk in Tris-buffered saline containing 0.05% Tween-20 (TTBS) (1h, RT). After blocking, membranes were washed and incubated overnight at 4°C with gentle agitation in the presence of specific primary antibodies: mouse anti-GFP 1:4000 (Santa Cruz Biotechnology, #sc-9996) or mouse anti-HA 1:3000 (Sigma-Aldrich, #H3663). After washing, membranes were incubated (2h, RT) with HRP-conjugated secondary antibodies anti-mouse 1:80000 (Sigma-Aldrich, #A9044). Signal detection was carried out by chemiluminescence using a ChemiDoc XRS+ system (BioRad) with either Clarity ECL Western Blotting Substrate (BioRad, #170-5060) or SuperSignal West Atto Ultimate Sensitivity Substrate (Thermo Scientific, #A38555). Exposure times varied from a few seconds up to 10 min, ensuring only unsaturated images were analyzed. After immunodetection, membranes were stained with Coomassie R-250 to verify equal protein loading. Input and IP samples were run in duplicate SDS-PAGE gels for GFP and HA detection. Each Co-IP assay was repeated three times with consistent results.

AlphaFold structural prediction and analysis

Structural models of the SILRX5^{LRR}–SIRALF5 and SILRX5^{LRR}–SIRALF10 complexes were generated using the AlphaFold 3 server (<https://alphafoldserver.com>) (Abramson et al., 2024). The full-length SILRX5^{LRR} sequence and the mature peptide sequences of SIRALF5 (ATKKYISYGALQKNSVPCSRRGASYNNCKPGAQANPYTRGCSAITRCRS) and SIRALF10 (GDVNYLSYKVTNDKPPICNEKVYGNCIGDKKPGQRPCTEYNNCKRSPD) were submitted as separate protein chain inputs. Five structural models were generated per complex; the

top-ranked model (model_0) was selected based on the highest per-residue confidence score (pLDDT) and interface predicted TM-score (ipTM). Models with ipTM > 0.6 were considered reliable predictions of the protein–protein interaction interface. For comparison, the ColabFold implementation of AlphaFold2-Multimer (alphafold2_multimer_v3) was also used as an alternative approach. Structural superposition of the tomato complexes with the reference LRX2–RALF4 structure (PDB: 6QXP) (Moussu et al., 2020) was performed by aligning the LRR domains (chain A) of each model using PyMOL (The PyMOL Molecular Graphics System, version 2.5, Schrödinger). Electrostatic surface potential was calculated using the APBS (Adaptive Poisson-Boltzmann Solver) plugin for PyMOL at pH 5.0 to simulate apoplastic conditions, with a solvent ion concentration of 0.15 M KCl and a grid spacing of 0.5 Å. The electrostatic potential was mapped onto the RALF peptide surface using a colour scale from −5 to +5 k_BT/e (red, negative; blue, positive), as previously described (Lee & Santiago, 2023; Moussu et al., 2020). All structural figures were rendered with PyMOL.

Statistic

Graphs and statistical analyses were performed using Prism 8.02 (GraphPad Software, www.graphpad.com), by applying one-way ANOVA followed by Dunnett’s multiple test (****P<0,0001; *P<0,01). Asterisks in figures indicate statistical differences between peptide treatments and mock. Experiments were repeated at least three times with similar results.

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Author contributions

Conceptualization: VGD; Investigation: JAM, MC, XW, PMR, AML, AS, IM, VGD; Writing-original draft: JAM, VGD; Writing- review & editing: all authors.

Competing interests

The authors declare no competing interests.

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Figures

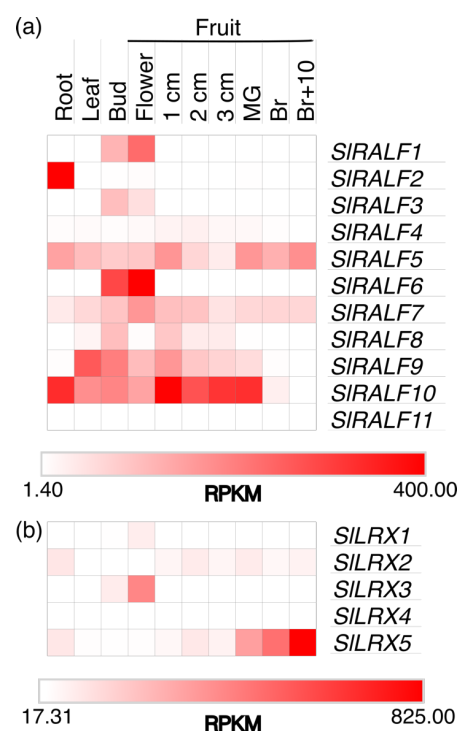


Figure 1: Expression pattern analysis of *SIRALFs* and *SILRXs*. (a) Heatmap of *SIRALF* expressions in a variety of tissues. (b) Heatmap of *SILRX* expressions in a variety of tissues. RPKM: reads per kilobase per million mapped reads. All of the data were acquired from the SGN RNA-seq database.

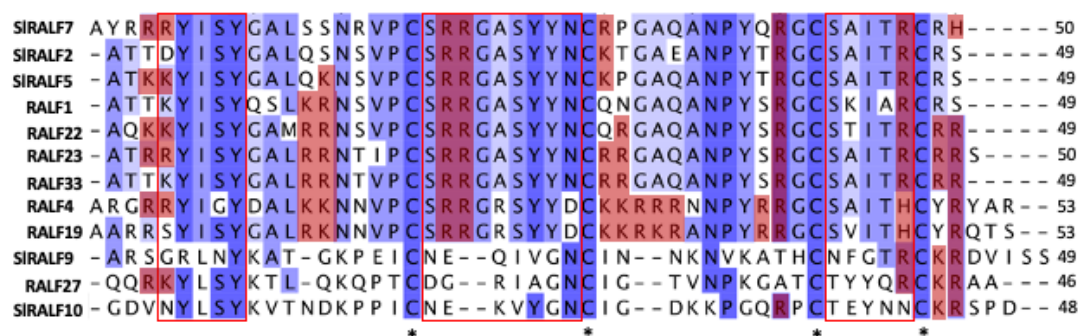


Figure 2: Alignment of the mature sequences of SIRALF5, SIRALF7 and SIRALF10 with their related tomato and Arabidopsis RALF members. Conserved residues are highlighted in different blue tones, whereas basic residues are shown in red. Motifs reported to mediate interactions with LLG proteins (N-terminal region) and LRX proteins (middle and C-terminal regions) proteins are enclosed in red boxes. Conserved Cys residues are indicated by black stars.

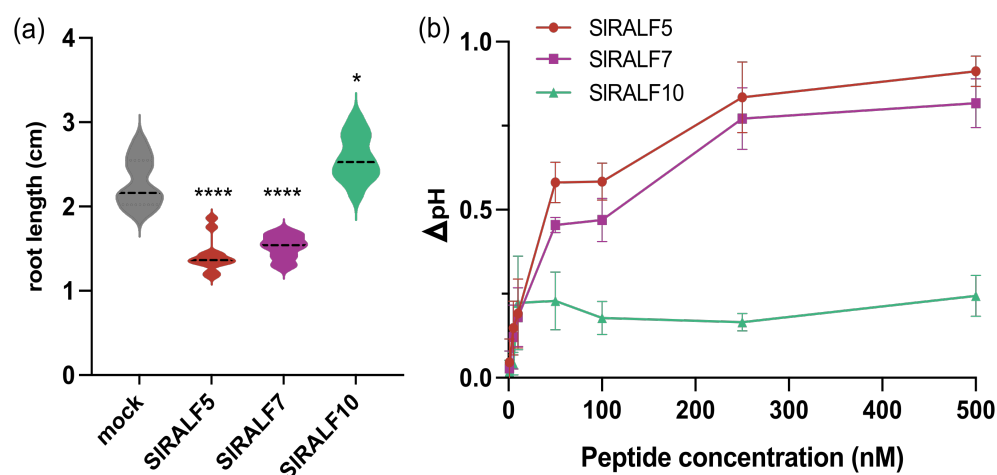


Figure 3: Effect of *S/RALF* peptides on root growth inhibition and extracellular alkalinization. (a) Primary root length of Arabidopsis 7-d-old seedlings grown in the absence (mock) or presence of 2 μ M *S/RALF* peptides ($n = 10$). Asterisks indicate significance, each treatment was compared with its corresponding mock: * (P -value < 0.01) and **** (P -value < 0.0001). (b) Dose-response curves showing Δ pH of tomato cell suspensions after addition of different *S/RALF* peptides concentration. Data points represent the mean $\pm$ standard error of three independent experiments.

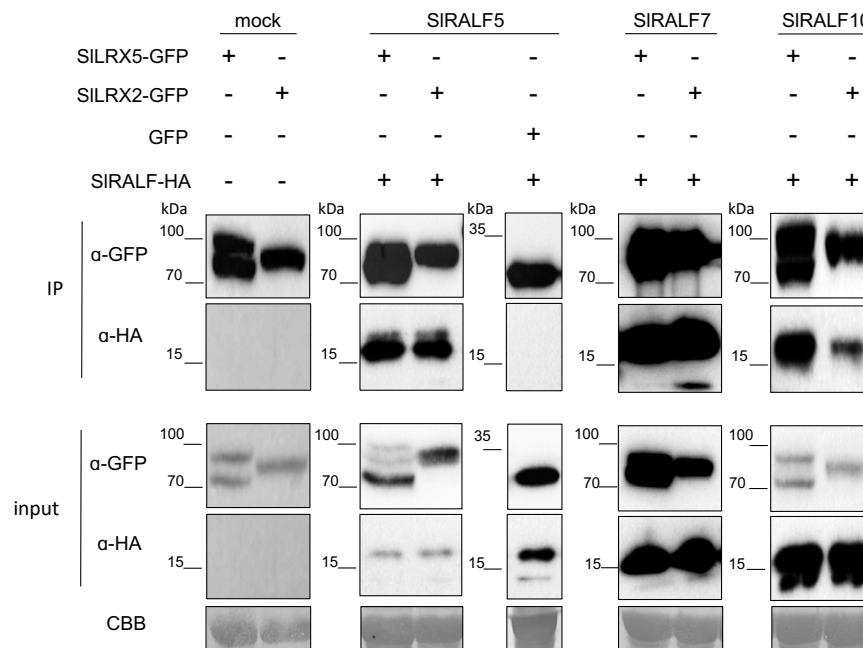


Figure 4: SIRALF5, SIRALF7 and SIRALF10 interact with SILRX2 and SILRX5. Western blot analysis of coimmunoprecipitated SIRALF-HA after immunoprecipitation of SILRX-GFP using antibody against GFP (α -GFP). Predicted sizes: SIRALF5-HA propeptide, 14,4 kDa, and mature 7,5 kDa; SIRALF7-HA propeptide, 16,7 kDa, and mature, 7,9 kDa; SIRALF10-HA propeptide, 12,9 kDa and mature, 7,6 kDa; SILRX2-GFP, 70,3 kDa; and SILRX5-GFP, 74,4 kDa. Equal loading was confirmed by Coomassie blue staining (CBB) of input samples. GFP- and HA-tagged proteins were detected with anti-GFP and anti-HA antibody, respectively.

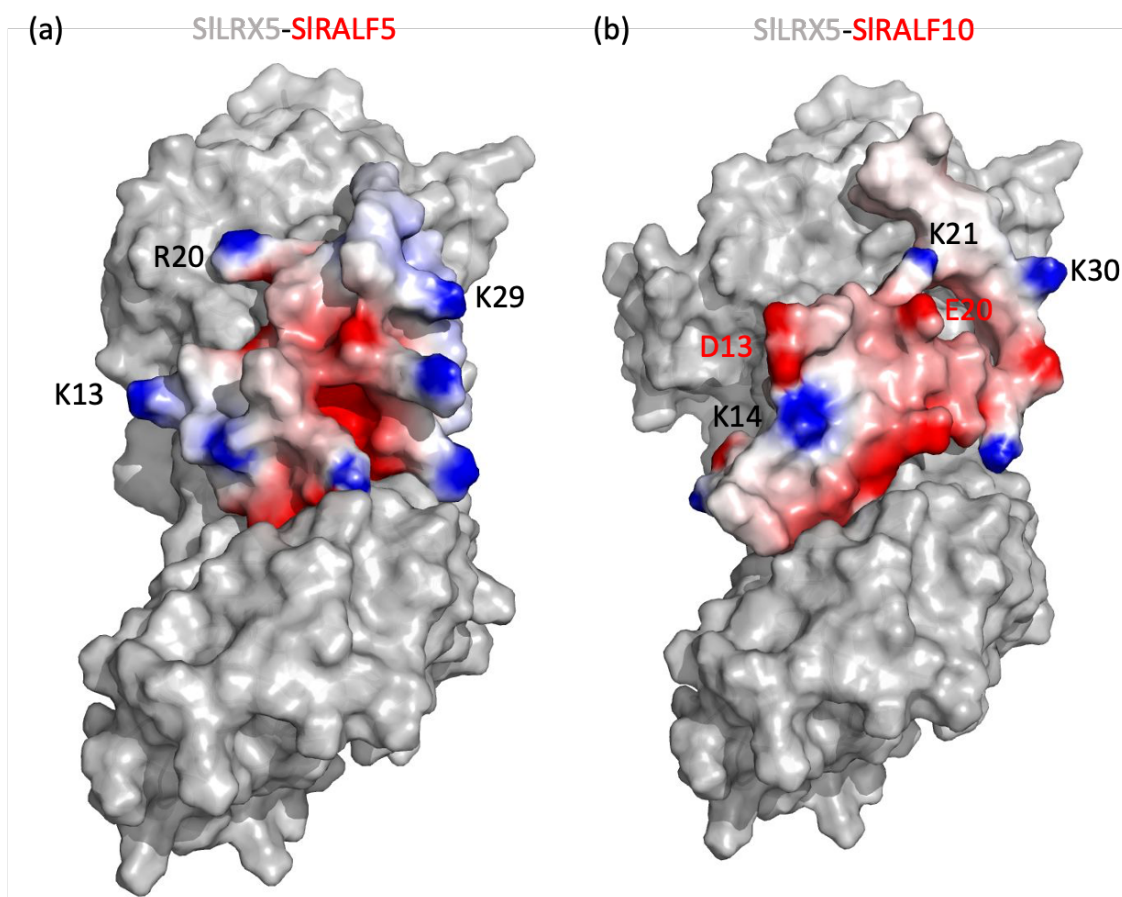
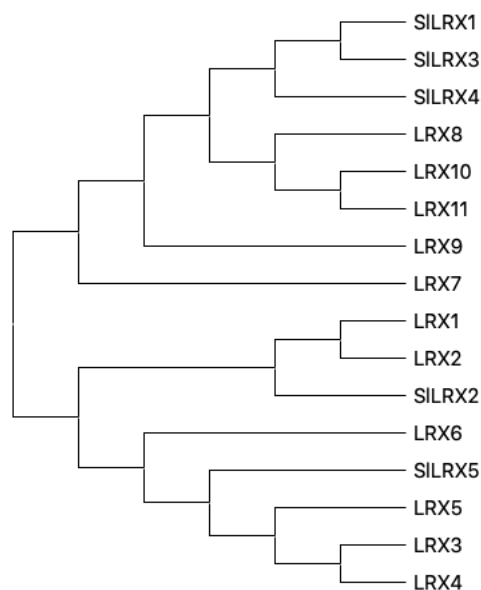
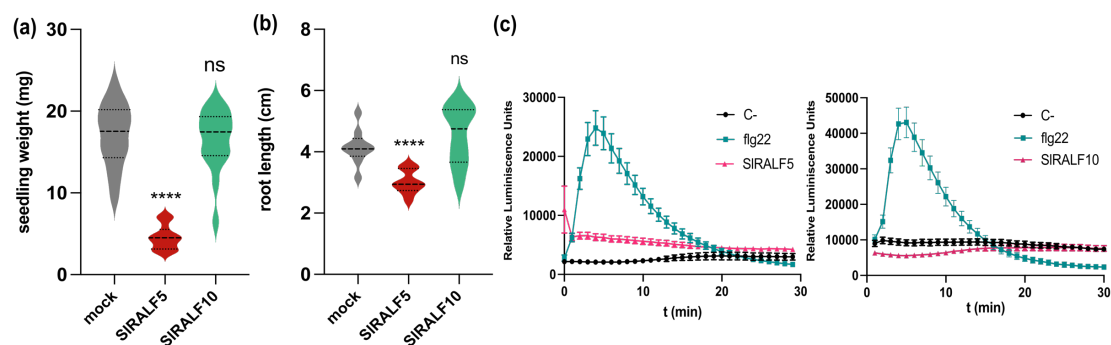


Figure 5: Exposed surface of SIRALF5 and SIRALF10 when bound to SILRX5. Electrostatic surface representation of SIRALF5 (a) and SIRALF10 (b) bound to SILRX5. Solven-accessible surface electrostatic potential has been calculated using the adaptative Poisson-Boltzmann solver (APBS) plugin (PyMOL) at pH 5. The potential is given with the negative (red) and positive (blue) contour levels in the range from -5.0 to +5.0 kBT. Details of the free residues are shown.

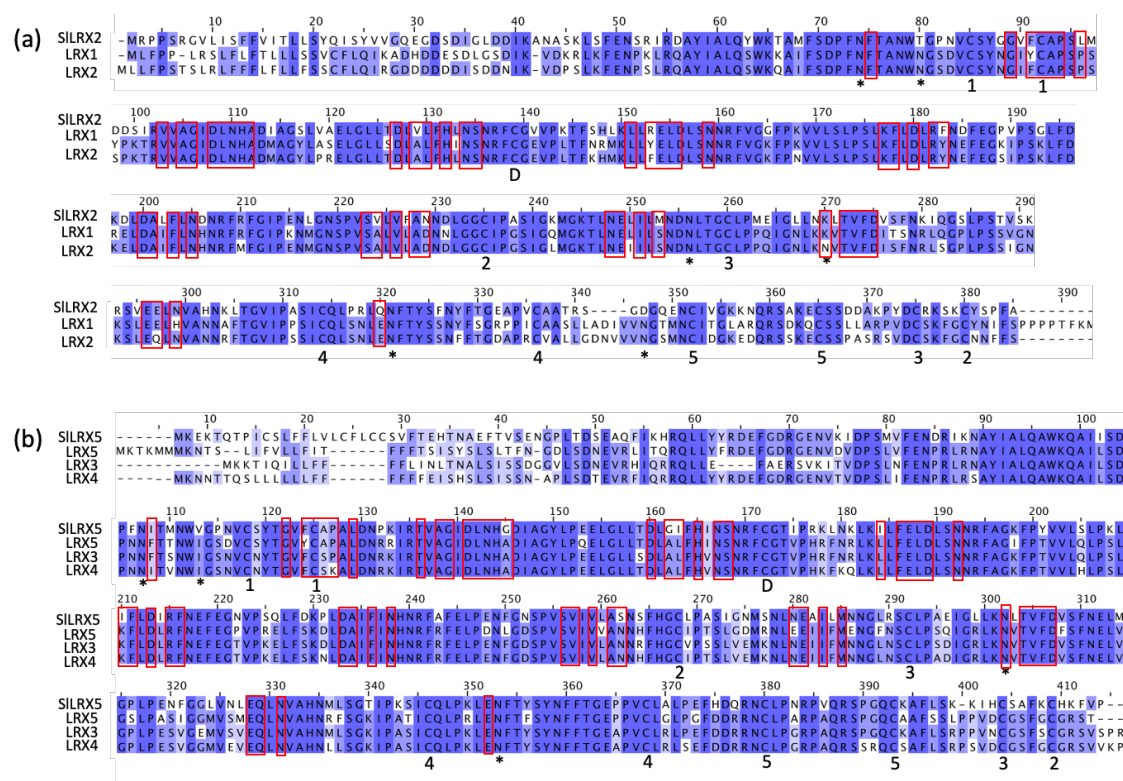
Supporting information



Supplementary Figure 1: Phylogenetic tree of LRX proteins based on an alignment of tomato and Arabidopsis sequences. The unrooted phylogenetic tree was constructed with ClustalOmega using the neighbor-joining method with 1000 bootstrap replicates and implemented in MEGA 5.0.



Supplementary Figure 2: Effect of *S/RALF* peptides on seedling weight, root growth inhibition and ROS production. (a) Fresh weight of 12-d-old *Arabidopsis* seedlings grown in the absence (mock) or presence of 2 μ M *SIRALF* peptide ($n = 10$). (b) Primary root length of tomato 7-d-old seedlings grown in the absence (mock) or presence of 2 μ M *S/RALF* peptides ($n = 10$). Asterisks indicate significance, each treatment was compared with its corresponding mock: ns (P -value $>0,05$) and ****(P -value $<0,0001$). (c) ROS production in *Arabidopsis* leaf discs treated with 4 μ M *SIRALF* peptide. Shown are the kinetics of one representative replicate.



Supplementary Figure 3: Sequence alignment of the LRR domain of SILRX2 (a) and SILRX5 (b) with their related Arabidopsis LRX members. Conserved residues are highlighted in different blue tones. Motifs reported to mediate interactions with RALF forming the binding pocket are enclosed in red boxes. Conserved Cys residues engaged in disulfide bonds with matching pairs are indicated by numbers. N-glycosylation sites are indicated by black stars.

Tables

Table S1: Expression data of *SIRALF* in different tissues.

Gene	Expression data from SGN-TFGB (RPKM)									
	Heinz_root	Heinz_leaf	Heinz_bud	Heinz_flower	Heinz_1cm_fr	Heinz_2cm_fr	Heinz_3cm_fr	Heinz_MG	Heinz_Br	Heinz_Br+10
<i>SIRALF1</i>	0.23	0.22	119.31	232.54	0.54	0	0.22	0	0	0
<i>SIRALF2</i>	417.99	1.37	3.38	5.64	1.34	1.14	0.83	2.3	1.32	1.74
<i>SIRALF3</i>	0.13	0	103.25	52.12	0.31	0	0	0.34	0	0
<i>SIRALF4</i>	6.25	9.26	6.4	10.11	20.89	24.37	13.59	12.15	5.04	0.59
<i>SIRALF5</i>	146.86	104.2	82.15	89.11	167.56	65.96	31.66	165.29	125.5	175.86
<i>SIRALF6</i>	0.66	0	289.73	559.44	0.77	0.19	1.02	0.79	0.13	0
<i>SIRALF7</i>	34.36	61.62	93.7	164.88	101.63	95.79	45	64.33	67.8	65.84
<i>SIRALF8</i>	0	18.08	103.27	6.13	88.32	32.46	32.87	2.34	0.4	0
<i>SIRALF9</i>	4.28	260.64	203.76	106.08	164.89	89.1	70.53	56.62	4.96	0.99
<i>SIRALF10</i>	331.78	178.03	191.55	145.57	396.55	271.85	319.43	326.12	26.37	1.33
<i>SIRALF11</i>	0	0	0	0	0	0	0	0	0	0

Table S2: Expression data of *SILRX* in different tissues.

Gene	Expression data from SGN-TFGB (RPKM)									
	Heinz_root	Heinz_leaf	Heinz_bud	Heinz_flower	Heinz_1cm_fr	Heinz_2cm_fr	Heinz_3cm_fr	Heinz_MG	Heinz_Br	Heinz_Br+10
<i>SILRX1</i>	0.14	0	20.1	75.17	0.09	0.03	0.35	0.23	0.11	0
<i>SILRX2</i>	95.48	2.18	7.13	3.73	49.82	81.44	49.82	80.32	43.51	57.3
<i>SILRX3</i>	1.1	0.21	77.98	400.34	0.28	0.06	1.62	0.82	0.35	0
<i>SILRX4</i>	0.7	0.37	4.57	18.05	0.3	0.37	0.18	0.37	0.55	0.96
<i>SILRX5</i>	93.17	23.79	20.8	25.12	42.37	89.77	57.94	322.71	471.42	825.65

Table S3: Amino acid sequence of the synthesized *SIRALF* peptides.

Peptide	Sequence of synthesized RALF
<i>SIRALF5</i>	ATKKYISYGALQKNSVPCSRRGASYYNCKPGAQANPYTRGCSAITRCRS
<i>SIRALF7</i>	AYRRRYISYGALSSNRVPCSRRGASYYNCRPGAQANPYQRGCSAITRCRH
<i>SIRALF10</i>	GDVNYLSYKVTNDKPPICNEKVYGNCIGDKKPGQRPCTEYNNCKRSPD

Table S4: Cloning primers for tomato genomic DNA.

Target gene	Primer name	Sequence
<i>SILRX2</i>	VGD012_attB1_LRX2_Fw	AAAAAGCAGGCTCCATGAGACCTCCTAGCCGG
<i>SILRX2</i>	VGD018_attB2_LRR2_Rv	AGAAAGCTGGGTCTGTAGCAAAAGGGCTATAACA
<i>SILRX5</i>	VGD013_attB1_LRX5_Fw	AAAAAGCAGGCTCCATGAAGGAGAAACTCAAACA
<i>SILRX5</i>	VGD019_attB2_LRR5_Rv	AGAAAGCTGGGTCCAAAACAGGAACAAATTTATGACA